

pressure transducer to prevent damage to the system and a second protection valve to control access to a fluid reservoir. The subroutines in the analysis program are burnt into the microprocessor.

A most preferred embodiment in accordance with this invention is a method for measuring blood platelet contractility. The method comprises the steps of preparing a retractometer according to this invention by applying adhesive to the surface of the inner flexible membrane to avoid slippage of clots. The adhesive can be any suitable substance, for example, collagen Type I suspension. The coated flexible membrane is then pressure conditioned by mounting it on a rubber stopper pierced by a hypodermic type needle attached to a two-way valve. A syringe is attached to one opening of the valve and a second needle is attached to a second opening of the valve, making certain that the reach of the two needles is identical.

Next, the membrane chamber is slightly pressurized, the valve to the syringe is closed, communication is opened to ambient fluid. In this manner, the inner and ambient pressures are allowed to equilibrate by siphoning. The fluid level inside the capillary is adjusted to "zero pressure" level.

The second step involves loading of the sample into the void created between the two chambers, surrounding and in contact with the outside surface of the flexible membrane chamber. A small amount of oil is added over the sample to avoid drying out. The sample is then allowed, or induced, to clot and the force of the clot retraction is measured in a pressure transducer and recorded.

Also contemplated by this invention is a method for automatically measuring a number of samples in a number of retractometers to determine the strength of platelet contractility. A first step requires calibration of the apparatus above. This entails the microprocessor reading all initial pressures in all retractometers sequentially by

opening each solenoid valve, opening the protection valve, measuring the voltage in the pressure transducer and storing the measured value in the temporary memory of the microprocessor. This process is repeated until all the initial pressure values are registered as target values for each of the retractometers. The second step adjusts the value of the hydraulics by opening the protection valve only and activating the pump until the target value is reached. The sample is then loaded into the retractometers, and clot formation is induced. The third step requires opening of the sample valve, measuring the pressure, and closing the sample valve, in that sequence. The measured values are then sent to a text file in a computer, and the new measured value for each retractometer becomes the next target value. This third step is repeated until all samples are measured. The entire process of measuring the clotted samples takes less than one minute.

The methods described here are useful in determining platelet activity. The ability to determine platelet activity, and contractile strength, is more specifically useful in determining viability of stored blood products. Determining forces of contractility is particularly useful in diagnosis or prognosis of various diseases in patients. Because each of the components associated with clotting is the result of a myriad of intermediate steps, clot retraction is an excellent candidate for “funnel detection” where with one simple measurement it is possible to unmask a vast array of pathological stages. Funnel detection methods are particularly important in population screening studies.

Still further embodiments and advantages of the invention will become apparent to those skilled in the art upon reading the entire disclosure contained herein.

## **BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1** shows Platelet interaction with surface immobilized collagen type I. RICM

images show platelets in a flow field. On a gray scale, black indicates a distance from the surface of 4-12 nm; white of > 20-30 nm. The time after initiation of the experiment is shown at the right side of each panel. For this experiment the platelet count was reduced to 10,000 platelets per  $\mu\text{l}$ .

5 **Figure 2** shows the evolution of an isolated thrombus at  $100\text{s}^{-1}$ . For the experiment shown here, development of a single thrombus was recorded. This image shows the thrombus once it is developed (>10 minutes) to observe the growth changes. Each image corresponds to the summation of a series of confocal images. Of notice is the peculiar growth pattern of the thrombus. Platelet deposition appears to occur in the  
10 downstream areas.

**Figure 3** is a graphic representation of the STL file generation. For the experiment shown in this figure, an isolated thrombus obtained with collagen spray was used. The wall shear rate was  $100\text{ s}^{-1}$  and the data presented correspond to images taken after 10 minutes of flow. The geometry reconstructed here corresponds to the thrombus shown  
15 in the early time of Figure 2

**Figure 4** is a photograph of the sterolithography model. The actual model was built to a scale of **300:1** and has a volume of  $3.44\text{ cm}^3$ .

**Figure 5** is a diagrammatic representation of two alternative designs for the retractometer of this invention. Top panel (A) is a design in which the individual  
20 retractometers are connected through a system of communicating vessels sharing a common pressure transducer. This design allows the simultaneous measurement of several samples. Bottom panel (B), is an alternate design having a clay plug closing the air filled capillary tube, which the operator “snaps” by bending it around the etching before starting the reading. The fluid inside the capillary then reaches the  
25 “zero level” corresponding to the hydrostatic pressure of the system. This design